# The karstic lake Iezerul Ighiel (Transylvania, Romania): its first limnological study

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**SUMMARY.** The present study represents the first limnological survey made in Lake Iezerul Ighiel, considering the most important biotic communities in the water column (phyto- and zooplankton) and from the bottom (phyto- and zoobenthos). No data was collected on ichthyofauna, since the species existing in the lake were introduced. The lake was sampled in 2014 in three seasons, from three sampling sites located in different regions of the water body, having different characteristics. Physical and chemical parameters were also analysed. The biotic communities considered for the study depicted a generally good ecological status of the lake. However, moderate organic pollution, together with initial phases of eutrophication were indicated by the algal and invertebrate communities considered for the present study, both planktonic and benthic.

**Keywords:** algae, benthic invertebrates, diversity, ecological status, microcrustaceans

### Introduction

Lake Iezerul Ighiel represents the largest karstic lake in Romania, located in the southern Trascău Mountains, on the upper course of the Ighiel River (Valea Iezerului); in the limestone plateau Ciumerna, at 940 m altitude (Gâştescu, 1971). Natural damming processes caused by landslides led to the formation of this oval shaped lake, 400 m long and 140 m wide, having a total volume of 225000 m³, a maximum depth of about 9 m and an average one of 4 m (Decei, 1981; Mihăiescu et al., 2012). The lake has a few surface tributaries, usually temporary and short brooks; but numerous underground springs are located in the central and northern parts of the lake. The water level fluctuates with minimums in summer and winter periods, the lake being recharged during spring and precipitation events by torrents (Pop and Măhăra, 1965; Duma, 2009). Natural dominant vegetation from the catchment

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area consists in beech forests, mixed with hornbeam and fir (*Fagus* sp., *Carpinus* sp. and *Abies* sp.). Pines and spruces were later planted to consolidate the slopes. At present, natural grasslands and transitional regions are also characteristic to the region (Pop and Măhăra, 1965; Mihăiescu *et al.*, 2012).

Lake Iezerul Ighiel is a Natural Reserve since 1969, now included in ROSCI0253: Trascău and ROSPA0087: the Trascău Mountains. The impacts that influence the lake are both natural (siltation) and human-induced (deforestation, tourism, poaching and domestic wastes). Since 1966 constant fish stocking is permitted in the lake, with various species like: *Salmo trutta fario, Oncorhynchus mykiss, Hucho hucho, Phoxinus phoxinus, Leuciscus cephalus, Cottus gobio,* even *Ctenopharyngodon idella* and *Aristichthys nobilis* (Decei, 1981), or *Cyprinus carpio* and *Carrassius auratus* at present.

The present limnological study focused on three important biotic communities characteristic to standing waters: phytoplankton, phytobenthos, planktonic microcrustaceans and benthic invertebrates.

Algae develop in all aquatic basins, each taxon having specific requirements and preferences to the environment (Brönmark and Hansson, 2005). Stenobiont forms represent accurate indicators of water quality in the ecosystems they inhabit (Bellinger and Sigee, 2010), that is why the Water Framework Directive 2000/60/EC states that planktonic and benthic algae should be used in assessing ecological status of water bodies

Zooplankton organisms occupy a central position in aquatic food webs, responding quickly to any changes in the status of their environment, natural or human-induced (Suthers and Rissik, 2009). Microcrustaceans (cladocerans and copepods) represent the most important group of animal plankton in terms of biomass, but also due to the fact that they occupy different guilds: filter-feeders, herbivores or predators. Even if they are not included in the stadardized methods of the Water Framework Directive 2000/60/EC, planktonic microcrustacean communities can offer valuable information on water quality, based on their taxonomical composition and community characteristics (Moss *et al.*, 2003; Haberman and Haldna, 2014).

Benthic invertebrates represent by far the most studied and diverse group of organisms in rivers, used to assess water quality (Giller and Malmqvist, 1998). In lakes, however, their importance decreases in favor of water column communities, due to the particularities of lentic habitats: greater depths, oxygen and light depletion near the bottom etc. Even though the Water Framework Directive requires assessments of macroinvertebrates in lakes, their use is extremely difficult (Moss *et al.*, 2003).

Previous studies from the area focused on geographical and abiotic parameters from the Lake Iezerul Ighiel (Pop and Măhăra, 1965; Decei, 1981; Duma, 2009; Mihăiescu *et al.*, 2012). Limnological studies considering biotic communities were not found, except for the inventory of stocked fish species from the lake (Decei, 1981). However, Negrea (1983) cited two cladoceran species from the karstic Lake

Ighiel - Trascău (*Daphnia rosea* and *Pleuroxus truncatus*), while Damian-Georgescu (1963, 1966, 1970) cited three copepod species *Eucyclops serrulatus*, *Acanthodiaptomus denticornis* and *Canthocamptus staphylinus*.

Thus, the aim of the present study was: (1) to characterize Lake Iezerul Ighiel from the point of view of three biotic communities inhabiting standing waters: algae, microcrustaceans and benthic invertebrates, considering their taxonomic composition, structure and diversity; (2) to assess the ecological status of Lake Iezerul Ighiel, reflected by the three biotic communities under study, since human impacts should be generally low in the area.

### Materials and methods

The samples were collected in 2014 from three sampling sites, in 10<sup>th</sup> of May, 15<sup>th</sup> of August and 1<sup>st</sup> of November 2014 (Figs. 1 and 2; Table 1). The sampling locations were chosen to be different, site 2 was sampled by boat, while the other two by wading, from the lake banks. Only benthic invertebrates were collected at site 3, due to the shallow water depth. The main characteristics of the sampling sites were depicted in Table 1.

The following physical and chemical parameters were recorded in the field or were measured subsequently in the laboratory: water temperature, dissolved oxygen (measured *in situ* with the portable meter YSI 52), pH and water conductivity (measured in the laboratory).

Plankton was collected using a 20-40 μm mesh size net in case of algae and a 50-55 μm mesh size one for microcrustaceans. Benthic algae were sampled by scraping the hard substratum or collecting the sediment using a pipette, while invertebrates were collected using a 250 μm mesh net for qualitative samplings. All samples were preserved in the field in 4% formaldehyde. Identifications were made to the species level in case of algae (Krammer and Lange-Bertalot, 1986, 1988, 1991; Ettl, 1983) and microcrustaceans (Negrea, 1983; Damian-Georgescu, 1963; 1966; 1970; Einsle, 1993; Janetzky *et al.*, 1996), and to different taxonomical levels in case of benthic invertebrates (Sansoni, 2001).

Relative abundance for planktonic and benthic invertebrates, expressed in percentages, represented a usefull tool in order to illustrate the structure of their communities. In order to compare diversities in different microcrustacean samples, diversity profiles were made, using a method that defines a family of diversity indices, dependent upon a single continuous parameter: the so-called Renyi index, which depends upon a parameter alpha (Tothmeresz, 1995). On the diversity ordering plot, the curve on top represents the most diverse community, while intersecting diversity profiles are non-comparable.

Algal communities have been long used to assess the ecological status of their environment, in terms of organic pollution, saprobity and trophicity. To assess the trophic state of the lake, the following indices were used in the present study: alpha-eutrophicity index, beta-eutrophicity index (Oltean, 1977); Nygaard compound index (1949), the Q index of eutrophy (Järnefelt, 1951) and the diatom index (Stockner, 1972). The saprobic indicator values of certain algal species were considered, following Rott (1997), Hindak (1978), Sládeček (1973). The organic pollution index (Palmer, 1969) was also used.



**Figure 1.** Location of Lake Iezerul Ighiel and the three sampling sites considered for the present study



**Figure 2.** View towards sampling site 1 in spring (left) and autumn (right), showing drastic drops in water level

The indication values of the most abundant microcrustaceans were considered. The species were sorted based on their frequency (the percentage of samples with the species) and on their average abundance in the samples. Cosmopolitan species were excluded, thus only species with indication values were selected, according to Sládeček (1973), Damian-Georgescu (1963, 1966, 1970), Negrea (1983).

Table 1.

Main characteristics of the three sampling sites considered for the present study
(SP - spring; SU - summer; AU - autumn; al - algae; mc - microcrustaceans;
bi - benthic invertebrates)

Site	Site	Date of sampling	GPS	Samples	Location of sampling	Characteristics
1	SP1 SU1 AU1	10.05.2014 15.08.2014 01.11.2014	N46°10'51.1" E23°21'57.2"	al; mc	- north-eastern part of the lake, near the touristic cottage - samples taken from the cottage pier in spring and summer, and from the lake banks in autumn - maximum depth of sampling: 1 - 1.5 m	- highly variable water levels: extremely high in spring (when surplus water was channeled on a tributary) and extremely low level in autumn, due to severe drought - substratum near the banks: rocks, silt, no submerged macrophytes
2	SP2 SU2 AU2	10.05.2014 15.08.2014 01.11.2014	N46°10'47.2" E23°21'48.4"	al; mc; bi	- south-western part of the lake, opposite to site 1; a tributary enters the lake there - near the maximum depth region of the lake - maximum depth of sampling: 3m for boat samples; 1 m for wading samples	- variable water levels: spring samples collected from the lake banks included a flooded area, covered with herbaceous vegetation (usually above water levels) - substratum near the banks: rocks, silt, submerged macrophytes
3	SP3 SU3 AU3	10.05.2014 15.08.2014 01.11.2014	N46°10'57.9" E23°21'44.3"	bi	- north-western part of the lake - maximum depth of sampling: 1 m for wading samples	- variable water level: spring samples collected from the lake banks included a flooded area, covered with herbaceous vegetation (usually above water levels) - substratum near the banks: rocks, silt, submerged macrophytes

Biotic indices based on zooplankton species are rare. The one chosen for this paper represents the ratio between large cladocerans (C<sub>1</sub>) and the density of all cladoceran species (C<sub>t</sub>) (Moss *et al.*, 2003). The values of this index indicate five water quality classes, according to the Water Framework Directive: when the values are lower than 0.2, the water quality is bad or poor; when the values vary between 0.2 and 0.5, the water quality is moderate; if they exceed 0.5, the water quality is good or high. The explanation resides in the fact that there is a greater proportion of large cladocerans in lakes at high ecological status, finding refuges from fish predation among the plant communities (Moss *et al.*, 2003).

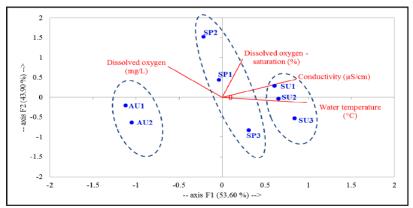
Multivariate analyses were used to visualize and interpret the data. Principal Component Analysis (PCA) was used for the physical and chemical parameters, due to its ability to project the data on a two dimensional map and to identify trends. Correspondence Analysis (CA) visualizes complex data, primarily data on categorical measurement scales, facilitating understanding and interpretation.

Multivariate analyses were performed using XLSTAT Version 2015.3.01.19199, while diversity profiles were made using PAST version 2.17c.

#### Results and discussion

## Physical and chemical parameters

Several physico-chemical parameters measured in the field or in the laboratory differentiated the three sampling locations according to their seasonal variation (Fig. 3). Thus, higher temperatures were recorded in summer 2014, a normal situation in the northern hemisphere, while lower dissolved oxygen saturations were recorded in autumn, probably due to the input of leaf material coming from the deciduous forest surrounding the lake, since high levels of organic carbon coincide with a lowering of dissolved oxygen concentrations.



**Figure 3.** Principal Component Analysis (PCA) biplot (axes F1 and F2: 97.50 %) for the three sampling sites and three seasons considered for the present study, and their aggregation based on physical and chemical parameters (abbreviations as in Table 1).

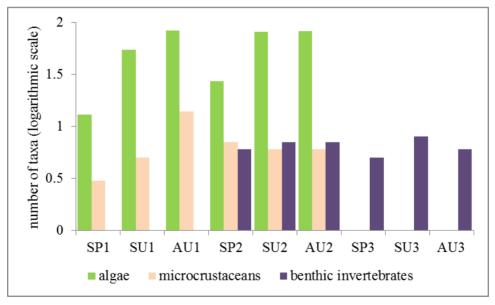
Conductivity values varied around 300  $\mu$ S/cm, except for autumn samples, where they decreased by half. pH was above 8, typical for karst areas.

According to Mihăiescu *et al.* (2012), the maximum concentration of Total Phosphorus (TP) found in a survey conducted in 2010 was 0.010 mg P /L, thus showing oligotrophic conditions from this point of view. However, Total Nitrogen (TN) values from the same study depicted a tendency towards eutrophication.

# Algal taxonomical survey

A total number of 152 algal taxa was identified at sampling sites 1 and 2 in 2014, belonging to the following phyla: Cyanophyta, Chlorophyta, Euglenophyta, Chrysophyta, Bacillariophyta and Dinophyta. Bacillariophyta dominated the algal communities, reaching a percentage of 69%, followed by Chlorophyta (24%), Chrysophyta (3%), Cyanophyta (2%), Euglenophyta (1%) and Dinophyta (1%).

Only planktonic samples were taken in spring 2014, while epiphytic and epilithic samples were added in summer and autumn 2014. Hence, the total number of taxa from the three seasons differed, from 36 in spring to 100 and 105 in summer and autumn, respectively (Fig. 4). Table 2 depicts the list of algal taxa identified in the study area, in summer and autumn 2014 plankton and periphyton considered together.



**Figure 4.** The number of algal, microcrustacean and benthic invertebrate taxa found in Lake Iezerul Ighiel (sampling sites as in table 1; logarithmic transformation based on log 10).

Table 2.
Algal taxa identified in two sampling sites from Lake Iezerul Ighiel
(sampling sites as in Table 1)

TAXA	SP1	SP2	SU1	SU2	AU1	AU2
Phylum Cyanophyta						,
Merismopedia glauca (Ehrenberg) Kützing 1845			+		+	
Oscillatoria limosa C. Agardh ex Gomont 1892			+	+	+	+
Oscillatoria redekei Goor 1918						+
Phylum Dinophyta						
Ceratium hirundinella (O.F.Müller) Dujardin 1841			+	+		
Phylum Euglenophyta						
Trachelomonas pulcherrima (Playfair) Popova 1955						+
Trachelomonas volvocina (Ehrenberg) Ehrenberg 1834						+
Phylum Chrysophyta						
Dinobryon bavaricum var. medium (Lemmermann)			+	+		
Krieger						
Dinobryon divergens O.E.Imhof 1887			+	+		
Epipyxis natans (Ruttner) Hilliard et Asmund					+	
Mallomonas crassisquama (Asmund) Fott 1962					+	+
Uroglena americana G.N.Calkins 1892		+	+	+	+	+
Phylum Bacillariophyta						
Achnanthes biasolettiana			+	+	+	+
Grunow in Cleve & Grunow 1880						·
Achnanthes flexella (Kützing) Brun 1880				+	+	+
Achnanthes lanceolata (Brébisson ex Kützing) Grunow		+	+	+		'
in Van Heurek 1880			'	'		
Achnanthes minutissima Kützing 1833		+	+	+	+	+
Amphipleura pellucida (Kützing) Kützing 1844			·	·		+
Amphora lybica Ehrenberg 1840			+		+	+
Amphora vyolca Efficience g 1840 Amphora ovalis (Kützing) Kützing 1844			'		+	+
Anomoeoneis vitrea			+	+	+	+
(Grunow) R.Ross in Patrick & Reimer 1966			'	'	'	'
Asterionella formosa Hassall 1850	+	+	+	+	+	+
Aulacoseira ambigua (Grunow) Simonsen 1979	'	'	'	'	+	'
Caloneis bacillum (Grunow) Cleve 1894					+	+
Caloneis silicula (Ehrenberg) Cleve 1894		+	+	+	+	+
Cocconeis placentula Ehrenberg 1838		+	'	'	+	+
Cyclotella antiqua W.Smith 1853		'	+	+	+	+
Cyclotella distinguenda Hustedt 1928		+	+	Τ-	+	+
Cyclotella iris Brun & HéribaudJoseph	+	+	+	+	+	+
	+	т	+	+	+	+
Cyclotella ocellata Pantocsek 1901	+		+	+		
Cyclotella planctonica Brunnthaler 1901					+	+
Cymatopleura solea (Brébisson) W.Smith 1851				+	+	+
Cymbella affinis Kützing 1844				+		
Cymbella amphicephala Näegeli in Kützing 1849			+		+	+
Cymbella aspera (Ehrenberg) Cleve 1894					+	
Cymbella cistula (Ehrenberg) O.Kirchner 1878			+	+	+	+
Cymbella cymbiformis C.Agardh 1830			+			+
Cymbella ehrenbergii Kützing 1844		+		+	+	+

## THE KARSTIC LAKE IEZERUL IGHIEL: ITS FIRST LIMNOLOGICAL STUDY

Table 2 continued

Cymbella lanceolata (C.Agardh) Kirchner 1878						+
Cymbella leptoceros (Ehrenberg) Kützing 1844				+		+
Cymbella minuta Hilse in Rabenhorst 1862		+	+	+	+	+
Cymbella silesiaca Bleisch in Rabenhorst 1864				+		
Cymbella simonsenii Krammer in Krammer &				+		
LangeBertalot 1985						
Cymbella tumida (Brébisson) van Heurck 1880				+		
Denticula tenuis Kützing 1844			+	+	+	+
Diploneis elliptica (Kutzing) Cleve 1894					+	
Diploneis oblongela (Nägeli ex Kützing) CleveEuler in				+		+
CleveEuler & Osvald 1922						
Diploneis petersenii Hustedt 1937			+	+	+	+
Eunotia arcus Ehrenberg 1837			+	+	+	+
Eunotia bilunaris (Ehrenberg) Schaarschmidt 1880					+	+
Fragilaria biceps Ehrenberg 1843					+	+
Fragilaria capucina var. vaucheriae (Kützing)					+	
LangeBertalot 1980						
Fragilaria crotonensis Kitton 1869	+	+	+	+	+	+
Fragilaria exigua (W.Smith) Lemmermann 1908			+	+		+
Fragilaria leptostauron var. martyi (HéribaudJoseph)		+				
LangeBertalot 1991						
Fragilaria parasitica (W.Smith) Grunow in van Heurck					+	
1881					•	
Fragilaria pinnata Ehrenberg 1843			+	+	+	+
Fragilaria ulna (Nitzsch) LangeBertalot 1980	+	+		+	+	+
Fragilaria ulna forma claviceps	'	'		'	+	+
1	+		+	+	+	+
Fragilaria ulna var. acus (Kützing) LangeBertalot 1980	+		'	'	'	1
Fragilaria ulna var. angustissima (Grunow) Krammer	Τ.					
& LangeBertalot 1991						
Fragilaria virescens Ralfs 1843				+	+	+
Frustulia rhomboides (Ehrenberg) De Toni 1891				+		
Frustulia vulgaris (Thwaites) De Toni 1891				+		
Gomphonema olivaceum (Hornemann) Brébisson 1838				+		
Gomphonema parvulum Kützing 1849				+	+	+
Gomphonema truncatum Ehrenberg 1832				+	+	+
Gyrosigma acuminatum (Kützing) Rabenhorst 1853					+	
Gyrosigma scalproides (Rabenhorst) Cleve 1894					+	+
Gyrosigma spenceri (Bailey ex Quekett) Griffith &		+	+	+	+	+
Henfrey 1856						
Hantzschia amphioxys (Ehrenberg) Grunow in Cleve &				+	+	+
Grunow 1880						
Melosira varians C.Agardh 1827					+	+
Meridion circulare (Greville) C.Agardh 1831	+					+
Navicula cincta (Ehrenberg) Ralfs in Pritchard 1861		+	+	+	+	+
Navicula concentrica Carter & BaileyWatts 1981				+		
Navicula cryptocephala Kützing 1844				+	+	+
Navicula cryptotenella LangeBertalot in Krammer &		+		+	+	
LangeBertalot 1985						
Navicula cuspidata (Kutzing) Kutzing 1844				+		+

Table 2 continued

					1 11010 2	commuca
Navicula cuspidata var. ambigua (Ehrenberg) Cleve 1894			+			+
Navicula elginensis (W.Gregory) Ralfs in Pritchard 1861				+		
Navicula gregaria Donkin 1861			+	+		
Navicula hasta Pantocsek 1892					+	+
Navicula laevissima Kützing 1844					+	
Navicula lanceolata Ehrenberg 1838				+		
Navicula menisculus Schumann 1867		+				
Navicula minuscula var. muralis (Grunow)			+			
LangeBertalot in LangeBertalot & Rumrich 1981						
Navicula nivalis Ehrenberg 1853			+	+		
Navicula pupula Kützing 1844			+	+	+	+
Navicula pygmaea Kützing 1849					+	+
Navicula radiosa Kützing 1844			+	+	+	+
Navicula trivialis LangeBertalot 1980				+	+	+
Navicula viridula (Kützing) Ehrenberg 1836				+		
Neidium affine (Ehrenberg) Pfizer 1871					+	
Neidium binodeforme Krammer in Krammer &			+	+		+
LangeBertalot 1985						
Neidium bisulcatum (Lagerstedt) Cleve 1894				+	+	+
Neidium dubium (Ehenberg) Cleve 1894		+		+		+
Neidium iridis (Ehrenberg) Cleve 1894				+		+
Nitzschia amphibia Grunow 1862		+		+		
Nitzschia dissipata (Kützing) Rabenhorst 1860					+	+
Nitzschia elegantula Grunow in van Heurck 1881					+	+
Nitzschia intermedia Hantzsch in Cleve & Grunow 1880		+	+	+	+	+
Nitzschia linearis W.Smith 1853					+	+
Nitzschia palea (Kützing) W.Smith 1856		+				
Nitzschia sinuata var. delognei (Kützing) W.Smith 1856				+		
Nitzschia subacicularis Hustedt 1922		+				
Pinnularia borealis Ehrenberg 1843			+			
Pinnularia maior (Kutzing) Cleve			+		+	+
Pinnularia microstauron (Ehrenberg) Cleve 1891				+		+
Rhoicosphenia abbreviata (C.Agardh) LangeBertalot 1980			+			
Stauroneis anceps Ehrenberg 1843				+	+	+
Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843						+
Stauroneis smithii Grunow 1860				+	+	
Surirella angusta Kützing 1844				+	+	+
Surirella brebissonii Krammer & LangeBertalot 1987						+
Surirella linearis W.Smith 1853				+		
Surirella spiralis Kützing 1844				+		
Tabellaria fenestrata (Lyngbye) Kützing 1844					+	
Tabellaria flocculosa (Roth) Kützing 1844	+					+
Phylum Cholorophyta						
Chloroccocum dissectum Korshikov 1953			+			
Chloromonas chlorogoniopsis		+				
(H.Ettl) Gerlof & H.Ettl in H.Ettl 1970						
Closteriopsis acicularis	+					
(Chodat) J.H.Belcher & Swale 1962						

Table 2 continued

Coelastrum astroideum De Notaris 1867 + + +  Coelastrum microporum Nägeli in A.Braun 1855 + +  Coenochloris fottii (Hindák) Tsarenko 1990 + +  Coenococcus planctonicus Korshikov 1953 + + +  Coenococcus polycoccus (Korshikov) Hindák 1977 + + +  Coleochaete divergens Pringsheim 1860 + + +
Coenochloris fottii (Hindák) Tsarenko 1990 + + + +  Coenococcus planctonicus Korshikov 1953 + + + +  Coenococcus polycoccus (Korshikov) Hindák 1977 + + +  Coleochaete divergens Pringsheim 1860 + +
Coenococcus planctonicus Korshikov 1953 + + + + + Coenococcus polycoccus (Korshikov) Hindák 1977 + + + + + Coleochaete divergens Pringsheim 1860 + + +
Coenococcus polycoccus (Korshikov) Hindák 1977 + + Coleochaete divergens Pringsheim 1860 + +
Coleochaete divergens Pringsheim 1860 + +
Cosmarium contractum var. minutum + + +
(Delponte) Coesel 1989
Cosmarium laeve Rabenhorst 1868 + +
Cosmarium regnellii Wille 1884 +
Cosmarium subgranatum (Nordstedt) Lütkemüller 1902 +
Cosmarium tenue W.Archer 1868 +
Crucigeniella apiculata (Lemmermann) Komárek 1974 + + + +
Crucigeniella rectangularis (Nägeli) Komárek 1974 +
Crucigenia triangularis Chodat 1902 +
Didymocystis bicellularis (R.Chodat) Komárek 1973 + + +
Gonatozygon brebissonii De Bary 1858 +
Monoraphidium griffithii (Berkeley) + +
KomárkováLegnerová 1969
Pandorina morum (O.F.Müller) Bory in Lamouroux, +
Bory de SaintVincent & Deslongschamps 1824
Pandorina smithii Chodat 1931 +
Pediastrum boryanum (Turpin) Meneghini 1840 + + +
Pediastrum boryanum var. longicorne Reinsch 1867 +
Pediastrum integrum Nägeli 1849 +
Pleurotaenium trabecula Nägeli 1849 + +
Scenedesmus acutus Meyen 1829 + + + +
Scenedesmus bicaudatus Dedusenko 1925
Scenedesmus dispar Brébisson 1868 +
Scenedesmus ecornis (Ehrenberg) Chodat 1926 +
Scenedesmus linearis Komárek 1974 +
Scenedesmus quadricauda (Turpin) Brébisson in +
Brébisson & Godey 1835
Schroederia ecsediensis Lemmermann 1898 +
Staurastrum paradoxum Meyen ex Ralfs 1848 + +
Tetraëdron muticum (A.Braun) Hansgirg 1888 +
Tetrastrum triangulare (Chodat) Komárek 1974 + + + +
TOTAL TAXA 13 27 54 81 83 82

A high number of taxa were cosmopolitan: Fragilaria virescens, Achnanthes minutissima, Cymbella minuta, Navicula cryptocephala etc. However, numerous species identified in Lake Iezerul Ighiel were known to have a subalpine or alpine distribution: Achnanthes flexella, Anomoeoneis vitrea, Caloneis bacillum, Cyclotella antiqua, Cyclotella planctonica, Cymbella affinis, Cymbella leptoceros, Cymbella simonsenii, Diploneis elliptica, Diploneis petersenii, Gomphonema acuminatum, Gonatozygon brebissonii, Navicula concentrica, Navicula nivalis, Neidium binodeforme,

Neidium bisulcatum. Moreover, several species had different preferences to physical and chemical parameters of the environment, like low water temperature: Cyclotella planctonica, Diploneis elliptica, Gomphonema acuminatum; higher values of pH: Cosmarium regnellii, Fragilaria crotonensis or high calcium content: Achnanthes biasolettiana, Achnanthes flexella, Cymbella ehrenbergii, Cymbella leptoceros, Cymbella simonsenii, Eunotia arcus, Meridion circulare, Surirella spiralis. True planktonic species were also found: Asterionella formosa, Fragilaria crotonensis, Ceratium hirundinella etc.

Three algal species are first cited for Romania, according to Cărăuş (2012): Epipyxis natans, Schroederia ecsediensis, Uroglena americana. Several other species can be considered rare in Romania, having only two previous citations: Coleochaete divergens, Cosmarium contractum var. minutum, Dinobryon bavaricum var. medium, Fragilaria virescens var. exigua, Navicula concentrica, Nitzschia sinuata var. delognei, Pandorina smithii

## Microcrustacean community structure

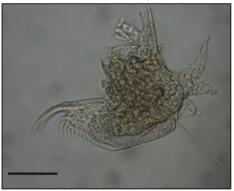
Sixteen taxa of microcrustaceans were identified in sites 1 and 2 in spring, summer and autumn 2014: 11 species of cladocerans and 5 species of cyclopoid, calanoid and harpacticoid copepods, together with immature copepod stages: copepodites and nauplii.

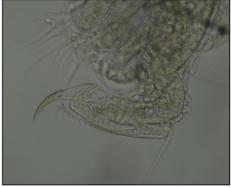
The number of taxa identified at site 1 ranged between 3 and 14 (Fig. 4, Table 3), probably due to the variable water level that changed drastically between the three sampling seasons. On the other hand, the number of taxa present at site 2 was more balanced throughout the year, probably due to the fact that the samples were taken by boat from a region where shore influences were decreased.

Seven taxa were only present at sample site 1 (Table 3), generally small-size cladocerans like *Alona guttata* or *Disparalona rostrata*, while *Daphnia* cf. *rosea* and immature copepod stages were the only microcrustaceans identified in all sampling occasions.

Several species were cosmopolitan, like the cladoceran *Chydorus sphaericus* or the harpacticoid copepod *Canthocamptus staphylinus*. Most of the species identified in the study area were planktonic, many preferring habitats with submerged macrophytes, while two were benthonic: *Ilyocryptus sordidus* and *Disparalona rostrata*.

The taxonomical identification of *Daphnia* sp. became especially problematic, since next to individuals belonging to *rosea* species, cited by Negrea (1983), several others, with different key taxonomical features were identified in all three seasons (Fig. 5). This particular genus is known to be one of the most difficult in terms of taxonomy, because of the co-existence of populations with intermediate morphologies, the presence of hybrids or different local races in large parts of its area (Petrusek *et al.*, 2008). Thus, all individuals were considered to be *D.* cf. *rosea*, while subsequent genetic studies should solve the taxonomical incertitudes.





**Figure 5.** Two different postabdomen morphologies in *Daphnia* cf. *rosea* identified in Lake Iezerul Ighiel in 2014: left - postabdomen typical to *D. rosea*, right - postabdomen typical to *D. galeata* (scale bar - 100 μm)

Diveristy profiles of the microcrustacean communities from sampling sites 1 and 2 were constructed, based on the sums of all individuals from each taxon, in order to compare them (Fig. 6). Surprisingly, sampling site 1 supported a greater diversity compared to the more balanced, macrophyte-covered sampling site 2 (with significant differences, t = 10.541, p < 0.01).

This is due to the fact that large-size taxa like Daphnia cf. rosea, Cyclops vicinus and Acanthodiaptomus denticornis inhabited the sampling site 2, since they could use the macrophytes as hiding place from visual predators like fish.

On the other hand, numerous small-size species were identified at site 1: *Alonella nana, Alona guttata, Chydorus sphaericus,* even *Attheyella crassa*. These species had no need to hide from visual predators, and probably they lost the competition with the larger *Daphnia* at sampling site 2.

The correspondence analysis in Fig. 7 shows this aggregation of small-size taxa in site 1 and large-size ones in site 2.

#### Benthic invertebrates

A total number of 15 benthic invertebrate groups were identified, 12 at the sampling site 2 and 9 at the sampling site 3. The highest number of taxa, at both sampling sites was recorded in summer 2014, probably due to the life cycle of the benthic invertebrate populations (Table 4).

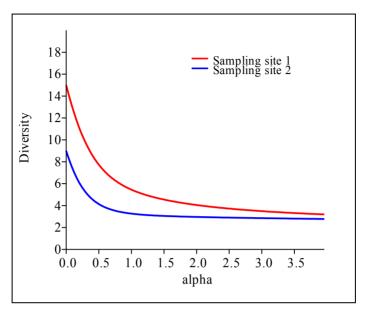
Three groups were present in all sampling occasions: Oligochaeta, Chironomidae and Acari-Hydrachnidia. On the other hand, other groups were present only at the sampling site 2: Hirudinea, Trichoptera, Coleoptera, Megaloptera, Stratiomydae, with low abundances, not exceeding 1.5%. They were probably carried into the lake by the tributary entering the water body near the location of the site.

Table 3.

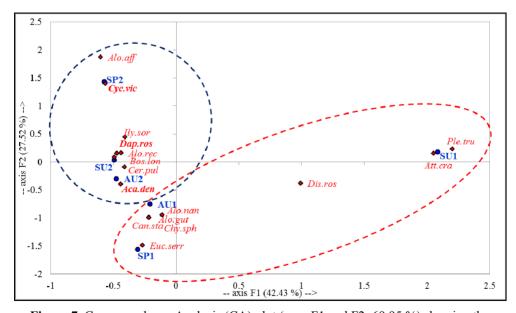
Microcrustaceans identified in the two sampling sites from Lake Iezerul Ighiel
(R - rare taxa; S - sporadic taxa; C - common taxa; ♀ - parthenogenetic cladoceran females and copepod females; † - gamogenetic cladoceran females;

♂ - males; sampling sites as in Table1)

TAXA	SP1	SP2	SU1	SU2	AU1	AU2					
(abbrevitations in brackets)											
Cl. Branchiopoda, Subcl. Phyllopoda, Ord. Diplostraca, Subord. Cladocera											
Alona affinis (Leydig 1860) (Alo.aff)	-	R,♀	-	-	-	-					
Alona guttata Sars 1862 (Alo.gut)	-	-	-	-	R,♀	-					
Alona rectangula Sars 1862	-	R,♀	-	-	R/S,	R,♂					
(Alo.rec)					₽,†,♂						
Alonella nana (Baird 1843) (Alo.nan)	S,♀	-	S,♀	R/S,♀	S/C,♀	R/S,♀					
Bosmina longirostris	-	R,♀	-	S,♀	R,♀	R,♀					
(O. F. Muller 1776) (Bos.log)											
Ceriodaphnia pulchella (Sars 1862)	-	R,♀	-	R/S,♀	R,♀,†	S,♀,†,♂					
(Cer.pul)											
Chydorus sphaericus	-	-	-	-	R/S,♀	-					
(O. F. Muller 1776) (Chy.sph)											
Daphnia cf. rosea Sars 1862 (Dap.ros)	S,♀	C,♀,♂	R,♀	C,♀,♂	S,♀,†	S/C,♀,†					
Disparalona rostrata (Koch 1841)	-	-	S,♀	-	S,♀,†,♂	-					
(Dis.ros)											
Ilyocryptus sordidus (Lievin 1848)	-	S,♀	-	-	R/S,♀	-					
(Ily.sor)											
Pleuroxus truncatus	-	-	S,♀	-	-	-					
(O. F. Muller 1785) ( <i>Ple.tru</i> )											
Cl. Maxillopoda, Subcl. Copepoda											
Acanthodiaptomus denticornis	-	-	-	S,♀, ♂	S/C,♀,♂	C,♀, ♂					
Kiefer 1932 (Aca.den)											
Attheyella crassa (Sars 1863)	-	-	R, ♂	-	S,♀, ♂	-					
(Att.cra)											
Canthocamptus staphylinus	-	-	-	-	R, ♀	-					
(Jurine 1820) ( <i>Can.sta</i> )											
Cyclops vicinus (Ulianine 1875)	-	S/C,♀,♂	-	R, ♂	R,♀, ♂	-					
(Cyc.vic)											
Eucyclops serrulatus proximus	R,♀	-	-	-	<b>S</b> ,♀, ♂	-					
(Lilljeborg 1901) (Euc.ser)											
Cyclopoid copepodites	R/S	S/C	R	R	S	R/S					
Calanoid copepodites	-	R	S/C	C	S	S/C					
Harpacticoid copepodites	-	-	-	-	R	-					
Nauplii	S	C	S/C	S/C	S	S/C					
TOTAL TAXA	3	7	5	6	14	6					



**Figure 6**. Diversity profiles for the microcrustacean communities from sampling sites 1 and 2, Lake Iezer Ighiel, in 2014



**Figure 7.** Correspondence Analysis (CA) plot (axes F1 and F2: 69.95 %) showing the aggregation of sampling sites with the number of individuals from each microcrustacean species (sampling sites as in Table 1; species as in Table 3)

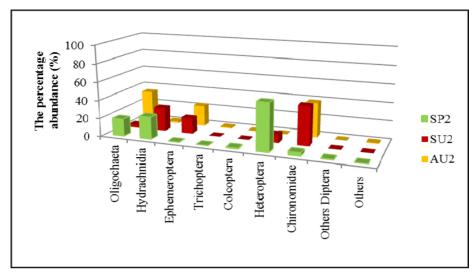
Table 4.

Benthic invertebrate taxa identified in two sampling sites from
Lake Iezerul Ighiel (sampling sites as in Table 1)

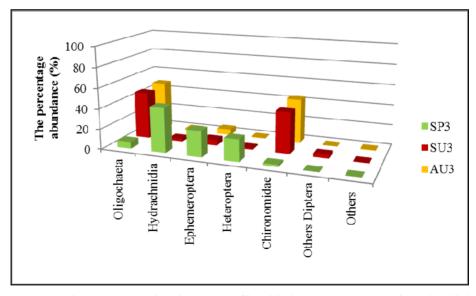
Taxa	SP2	SP3	SU2	SU3	AU2	AU3
Nematoda						+
Annelida, Oligochaeta	+	+	+	+	+	+
Annelida, Hirudinea					+	
Arthropoda, Chelicerata, Acari, Hydrachnidia	+	+	+	+	+	+
Athropoda, Hexapoda, Insecta, Ephemeroptera		+	+	+	+	+
Athropoda, Hexapoda, Insecta, Trichoptera					+	
Athropoda, Hexapoda, Insecta, Odonata			+	+		
Athropoda, Hexapoda, Insecta, Megaloptera					+	
Athropoda, Hexapoda, Insecta, Coleoptera	+					
Athropoda, Hexapoda, Insecta, Heteroptera	+	+	+	+		+
Athropoda, Hexapoda, Insecta, Diptera, Chironomidae		+	+	+	+	+
Athropoda, Hexapoda, Insecta, Diptera, Chaoboridae			+			
Athropoda, Hexapoda, Insecta, Diptera, Culicidae				+		
Athropoda, Hexapoda, Insecta, Diptera, Psychodidae				+		
Athropoda, Hexapoda, Insecta, Diptera, Stratiomydae	+					
TOTAL TAXA	6	5	7	8	7	6

Benthic invertebrate communities from the two sampling sites had a heterogeneous and dissimilar structure. Mayflies (Ephemeroptera) recorded high abundances during spring 2014 at sampling site 3, and during summer and autumn at site 2. Water mites (Hydrachnidia) reached the highest abundances in spring at both sampling sites, while at site 2 they maintained high numbers during summer too. True bugs (Heteroptera) dominated the benthic communities in spring at both sampling sites. Chironomids recorded higher abundances in summer and autumn in both sampling locations. Oligochaetes prevailed in autumn (Figs. 8 and 9).

Four water mite taxa (Acari - Hydrachnidia) were identified in the present study. The most frequent species was  $Limnesia\ konikei$ , known to be tolerant to high nutrient loads - up to 10 mg/L Total Nitrogen (Haaren and Tempelman, 2009). Four individuals  $(1 \circlearrowleft; 3 \circlearrowleft)$  belonging to  $Neumania\ deltoides$  were also found. This species occurred in waters strongly influenced by man in Netherlands (Smit and Hammen, 2000) or in eutrophic lakes in Italy (Cicolani and Di Sabatino, 1985). Two taxa typical for rivers were identified at sampling site 2:  $Hygrobates\ foreli\ (1 \hookrightarrow)$  and  $Lebertia\$ sp. (deutonymph), probably from the tributary flowing into the lake in that area.



**Figure 8.** The percentage abundance (%) of benthic invertebrate groups from the second sampling site (sampling sites as in Table 1, Others - Diptera: Chaoboridae, Stratiomidae; Others: Hirudinea, Odonata, Megaloptera)



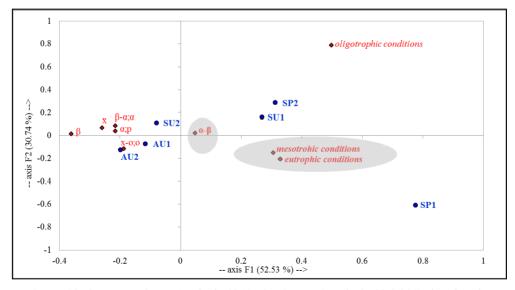
**Figure 9.** The percentage abundance (%) of benthic invertebrate groups from the third sampling site (sampling sites as in Table 1, Others - Diptera: Culicidae, Psychodidae; Others: Nematoda, Odonata)

# Ecological status of the lake, as indicated by biotic communities

The structure of <u>algal communities</u>, both phytoplankton and periphyton, were used to assess the ecological status of Lake Iezerul Ighiel, considering the saprobity and organic pollution characteristic to the system, but also water trophicity.

In terms of organic pollution, eight out of the 20 indicator algal genera, included in the Palmer genus index (Palmer, 1969) were identified in Lake Iezerul Ighiel: *Cyclotella, Gomphonema, Melosira, Navicula, Nitzschia, Oscillatoria, Pandorina* and *Scenedesmus*. Similarly, 7 indicator species for organic pollution, from a total of 20, were found in the two sampling sites considered for the present study: *Gomphonema parvulum, Melosira varians, Navicula cryptocephala, Nitzschia palea, Oscillatoria limosa, Pandorina morum* and *Scenedesmus quadricauda*. The values of the organic pollution index, both at genus and at species levels (19 and 20, respectively) indicated moderate organic pollution in the water.

These findings were supported by the number of algal taxa with saprobic indicator values, identified at each sampling site (Fig. 10). Even if no trends were visible in terms of clear relationships between indicator taxa and one sampling site or sampling season in particular, most algal taxa indicated oligosaprobic -  $\beta$  mesosaprobic waters (Fig. 10), meaning clean to slightly polluted waters in terms of the quantity of decomposing organic matter existing in the system.



**Figure 10.** Correspondence Analysis (CA) plot (axes F1 and F2: 83.26 %) showing the aggregation of sampling sites with the number of algal taxa with indicator value (sampling sites as in Table 1; x - xenosaprobic; o - oligosaprobic; β: β - mesosaprobic taxa; α: α - mesosaprobic taxa; p - polysaprobic taxa; intermediate classes also depicted)

The trophic state of the ecosystem was assessed using several indices based on algal communities. According to Oltean (1977), alpha-eutrophic conditions were detected in spring 2014, because Bacillariophyceae led to "water blooms" in the system; while beta-eutrophic ones were depicted in summer 2014, due to the fact that Dinophyta was responsible for the "water blooms" that season. The values of the indices (0.4668 and 0.3644, respectively), together with the values of two other indices: 4.5 for Nygaard index and 2.28 for Stockner index showed an early phase of eutrophication in Lake Iezerul Ighiel. Similar results were depicted in Fig. 10, where mesotrophic-eutrophic conditions were placed at the center of the CA plot. Thus, the highest number of algal taxa with indicator value showed increasing nutrient loads in the lake, coming probably from the touristic and fish-stocking activities in the area.

Five microcrustacean species were considered for the assessment of water quality in Lake Iezerul Ighiel, based on their high frequency and abundances at the sampling occasions. Thus, the first two dominant taxa, Daphnia cf. rosea and Acanthodiaptomus denticornis indicated oligosaprobic conditions. However, the other three species, Bosmina longirostris, Cyclops vicinus and Ceriodaphnia pulchella indicated oligosaprobic to  $\beta$ -mesosaprobic conditions, and mesotrophic towards eutrophic waters.

The biotic index represented by the ratio between the number of large cladocerans  $(C_l)$  to all cladoceran species  $(C_t)$  (Moss *et al.*, 2003) revealed a general good condition (0.72 - high water quality).

The ratio between calanoid and cyclopoid copepods can give valuable information on the trophic state of the water body. Nicholls and Tudorancea (2001) correlated the disappearance of calanoid species to oxygen concentration depletion and significant nutrient loads in the system. In Lake Iezerul Ighiel however, the ratio between calanoids (*Acanthodiaptomus denticornis*) and cyclopoids (*Cyclops vicinus*; *Eucyclops serrulatus proximus*) had a general value of 5:1, thus showing good trophic conditions.

Benthic invertebrate communities were difficult to use in assessing the ecological status of the lake. However, generally speaking, high abundances of pollution intolerant groups like Ephemeroptera and Trichoptera indicated a good water quality in Lake Iezerul Ighiel at sampling sites 2 and 3.

#### Conclusions

The present study focused on the most important planktonic and benthic communities from Lake Iezerul Ighiel. Both phytoplankton and periphyton were well represented in the lake, comprising more than 150 taxa with different indicator values of water saprobity and/or trophic state. Three species were first cited for Romania.

Microcrustacean communities were diverse and relatively balanced, with smaller taxa inhabitting the shallower, macrophyte-free regions of sample site 1 and with larger taxa concentrated on the area covered in aquatic macrophytes from sample site 2, where they could escape visual predators like fish.

Benthic invertebrate communities were also well represented near the banks of the lake. They were heterogeneous, with different groups prevailing in different periods of time.

The ecological status of Lake Iezerul Ighiel was assessed based mainly on algal and microcrustacean communities, that provided the same information. Thus, the lake was characterized by a moderate organic pollution, with low quantities of decomposing organic matter in the system. Both algal and microcrustacean species depicted initial eutrophication conditions in the lake.

To conclude, the ecological status of Lake Iezerul Ighiel was not oligotrophic and oligosaprobic, as expected in a relatively isolated, mountainous lake, but with moderate organic pollution and increasing nutrient loads, probably due to tourism and fish-stocking.

**Acknowledgements.** Thanks are given to RNP Romsilva for allowing access by boat on the lake and to our colleague, Rahela Carpa, PhD, for processing the pH and conductivity samples.

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